

# Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort

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Epilepsy is common in early childhood. In this age group it is associated with high rates of therapy-resistance, and with cognitive, motor, and behavioural comorbidity. A large number of genes, with wide ranging functions, are implicated in its aetiology, especially in those with therapy-resistant seizures. Identifying the more common single-gene epilepsies will aid in targeting resources, the prioritization of diagnostic testing and development of precision therapy. Previous studies of genetic testing in epilepsy have not been prospective and population-based. Therefore, the population-incidence of common genetic epilepsies remains unknown. The objective of this study was to describe the incidence and phenotypic spectrum of the most common single-gene epilepsies in young children, and to calculate what proportion are amenable to precision therapy. This was a prospective national epidemiological cohort study. All children presenting with epilepsy before 36 months of age were eligible. Children presenting with recurrent prolonged (>10 min) febrile seizures; febrile or afebrile status epilepticus (>30 min); or with clusters of two or more febrile or afebrile seizures within a 24-h period were also eligible. Participants were recruited from all 20 regional paediatric departments and four tertiary children's hospitals in Scotland over a 3-year period. DNA samples were tested on a custom-designed 104-gene epilepsy panel. Detailed clinical information was systematically gathered at initial presentation and during follow-up. Clinical and genetic data were reviewed by a multidisciplinary team of clinicians and genetic scientists. The pathogenic significance of the genetic variants was assessed in accordance with the guidelines of UK Association of Clinical Genetic Science (ACGS). Of the 343 patients who met inclusion criteria, 333 completed genetic testing, and 80/333 (24%) had a diagnostic genetic finding. The overall estimated annual incidence of single-gene epilepsies in this well-defined population was 1 per 2120 live births (47.2/100 000; 95% confidence interval 36.9–57.5). *PRRT2* was the most common single-gene epilepsy with an incidence of 1 per 9970 live births (10.0/100 000; 95% confidence interval 5.26–14.8) followed by *SCN1A*: 1 per 12 200 (8.26/100 000; 95% confidence interval 3.93–12.6); *KCNQ2*: 1 per 17 000 (5.89/100 000; 95% confidence interval 2.24–9.56) and *SLC2A1*: 1 per 24 300 (4.13/100 000; 95% confidence interval 1.07–7.19). Presentation before the age of 6 months, and presentation with afebrile focal seizures were significantly associated with genetic diagnosis. Single-gene disorders accounted for a quarter of the seizure disorders in this cohort. Genetic testing is recommended to identify children who may benefit from precision treatment and should be mainstream practice in early childhood onset epilepsy.

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## Introduction

It is estimated that the lifetime prevalence of epilepsy is 7.60 per 1000 individuals (Fiest *et al.*, 2017) and that 50–65 million individuals are affected worldwide (Ngugi *et al.*, 2010; Fiest *et al.*, 2017). Epilepsy incidence is age-dependent, with the highest incidences (>60 per 100 000) found in those under the age of 5 years and those over the age of 65 years (Hauser *et al.*, 1993). Even in high resource health systems seizure control is not achieved for one-third of subjects with epilepsy (Kwan and Brodie, 2000).

Children presenting with epilepsy before the age of 3 years experience a high burden of cognitive and behavioural comorbidity (Berg *et al.*, 2008). Comorbidities are more prevalent among children who develop drug-resistant seizures (Wirrell *et al.*, 2012) and those with a high seizure burden (Berg *et al.*, 2012; Wilson *et al.*, 2012). The concept of the 'developmental and epileptic encephalopathy' (DEE) recognizes that in children presenting with early-onset epilepsy, neurodevelopmental comorbidity may be attributable to both the underlying cause and to the detrimental effects of uncontrolled epileptic activity (Scheffer *et al.*, 2017). Single-gene causes of childhood-onset epilepsy, such as Dravet syndrome due to *SCN1A* mutations, typify this concept. Families affected by, and clinicians treating, such epilepsies strive for therapies that more precisely target the syndrome and/or the underlying disease mechanisms, in the hope that seizure control and developmental comorbidity can be simultaneously addressed. Precision therapy approaches have driven randomized therapeutic trials including stiripentol (Chiron *et al.*, 2000) and cannabidiol (Devinsky *et al.*, 2017) in Dravet syndrome, and quinidine in *KCNT1*-associated epilepsy (Mullen *et al.*, 2018).

The application of next generation sequencing (NGS) technology has facilitated a fundamental change in aetiological diagnosis of epilepsy. When cohorts of children with a suspected genetic cause are tested using NGS, between 18% and 48% receive a diagnosis. The variation in yield may be explained by differences in case inclusion, testing methodology, and number of genes tested (Lemke *et al.*, 2012; Appenzeller *et al.*, 2014; Helbig *et al.*, 2016; Trump *et al.*, 2016; Lindy *et al.*, 2018). Patients included in such studies may have been selected on the basis of the nature of the epilepsy and/or a suspected genetic aetiology. Therefore, these studies may misrepresent the incidence and phenotypic spectrum of the single-gene epilepsies. To appreciate the scope for genetically-guided precision medicine in childhood-onset epilepsy, and to prioritize therapy development, we must understand the epidemiology. This demands a prospective population-based approach to genetic testing.

## Materials and methods

### Cohort recruitment

Participants were recruited from all 20 regional paediatric departments and four tertiary children's hospitals in Scotland, from 8 May 2014 to 7 May 2017. Only children who met the inclusion criteria during this period, and who were under 36 months of age, were included.

Inclusion criteria were any of: (i) child receiving a new diagnosis of epilepsy (recurrent unprovoked seizures); (ii) child presenting with an episode of febrile or afebrile status epilepticus (seizure >30 min); (iii) child presenting with two or more febrile or afebrile epileptic seizures within a 24-h period; and

(iv) child presenting with a second prolonged (>10 min) febrile seizure, over any time period.

Patients were excluded if an aetiology that would fully explain seizures was identified either prior to or at first presentation with seizures. Examples of such aetiologies were meningitis, hypoxic ischaemic encephalopathy in the neonate, or focal seizures in an infant with a perinatal stroke.

Children presenting with prolonged and clustering febrile seizures were included because in certain genetic epilepsies, including those associated with *SCN1A*, *PCDH19* and *PRRT2* variants, epilepsy can be preceded by febrile seizures (Brunklaus *et al.*, 2012; Higurashi *et al.*, 2013; Ebrahimi-Fakhari *et al.*, 2015). Our aim was to optimize case identification and avoid any delay in genetic diagnosis. Early genetic diagnosis may inform treatment and potentially alter disease course (Brunklaus *et al.*, 2013; Lange *et al.*, 2018).

Maximum case ascertainment was ensured by weekly e-mail reminders throughout the study period to the eight paediatric neurophysiology departments, a link clinician in each of the 24 centres and all 17 epilepsy specialist nurses in Scotland. Research nurses throughout Scotland reviewed admissions to intensive care and high dependency units; and a national continuing education program maintained the profile of the study.

We are not aware of any private medical services in Scotland where young children would present with seizures, and given the geographical location of hospitals in the border regions of England and Scotland we would expect that children in Scotland would access both paediatric and paediatric neurology services from hospitals of the Scottish National Health Service (NHS).

Denominator data for births over the study period were taken from National Records of Scotland birth records (National Records of Scotland, 2018). Incidence estimates were rounded to three significant figures and 95% confidence intervals (CIs) were calculated using the Poisson distribution.

## Genetic testing

DNA was extracted from whole blood and tested on a custom designed 104 gene epilepsy panel (Supplementary material, part C) at the Scottish Genetic Epilepsy service in Glasgow, unless a genetic diagnosis had already been made through single-gene testing. Accelerated single-gene testing [Sanger sequencing and multiplex ligation probe amplification (MLPA)] of 10 genes (Supplementary material, part D) was offered, with clinicians advised to request these prior to panel testing if clinically indicated. The gene panel was designed to include the early onset childhood genetic epilepsies for which brain imaging was unlikely to give a diagnosis and for which there is evidence for specific therapeutic approaches. Genes to be included on the panel were selected by a team of clinicians (J.D.S., S.M.Z., S.J., D.T.P.) who reviewed the literature extensively. All potentially causative variants identified were validated through Sanger sequencing. Cases negative on the 104 gene panel with typical phenotypes for epilepsy related to *SCN1A*, *KCNQ2*, *SLC2A1*, *PCDH19*, *CDKL5*, or *MECP2* underwent dosage analysis through MLPA of the relevant gene. All variants of uncertain significance and likely pathogenic/pathogenic variants were discussed in the context of the clinical phenotype by a multidisciplinary team of paediatric neurologists, clinical geneticists and molecular geneticists. Variants were reported with reference to UK Association of

Clinical Genetic Science (ACGS) guidelines (Association for Clinical Genetic Science, 2017). Pathogenic and likely pathogenic results were considered diagnostic. Where DNA samples and/or phenotype details from other family members were considered relevant to variant interpretation these were requested. Chromosomal microarray studies were not routinely performed because results were not thought likely to guide therapeutic management.

## Clinical information

At the time of case recruitment, clinicians completed a structured proforma detailing clinical features and investigation findings (Supplementary material, part E). A panel of three paediatric neurologists reviewed clinical details of all cases to ensure eligibility criteria were met. A minimum of 12 months after initial presentation and 6 months after the recruiting clinician had been informed of the final genetic test result, clinicians completed a structured clinical follow-up questionnaire (Supplementary material, part F). For the purposes of this study, therapy-resistant seizures were defined as ongoing epileptic seizures (one or more seizure per 6 months) despite adequate trials of two or more appropriately chosen, appropriately dosed (or administered) and taken antiepileptic therapies (including non-drug therapies such as ketogenic diet and vagus nerve stimulation). The study was approved by the United Kingdom NHS National Research Ethics Service.

## Statistical analysis

IBM® SPSS® Statistics Version 24 was used to determine associations between clinical features and identification of a genetic cause. Only patients who had completed genetic testing were included in this analysis. Patients were defined as having completed genetic testing if either a diagnostic result was identified through accelerated single-gene testing, or gene panel testing was completed. Age of seizure onset was divided into four categorical groups: <6 months, 6–12 months, 12–24 months, and 24–36 months. Type of first seizure was divided into eight categories: febrile generalized (excluding status, which was defined as a seizure lasting >30 min), febrile focal (excluding status), febrile status (focal or generalized), afebrile focal (excluding status), afebrile generalized (excluding status), afebrile status (focal or generalized), afebrile unclassified, and infantile spasms. Univariate analysis was performed using Fisher's exact equation and multivariate analysis used a Hosmer-Lemeshow binomial regression model. Statistical advice was from Cunyi Wang of Glasgow University.

## Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary material.

## Results

The mean number of births per year in Scotland for the years 2011 to 2016 inclusive was 56 490, making the estimated denominator for this population 169 470. During

the 3-year study period, 343 children under the age of 3 years were recruited to this study.

## Phenotypes

Of 214 patients recruited because of a diagnosis of epilepsy, 101 (47%) presented with focal seizures, 42 (20%) with tonic-clonic seizures, 23 (11%) with myoclonic seizures, 22 (10%) with epileptic spasms, 11 (5%) with absence seizures, eight (4%) with generalized tonic seizures, three (1%) with atonic seizures, and four with unclassified seizures. Ninety-four patients were recruited because of febrile seizures, of whom 45 presented with febrile status, 36 with a cluster of two or more febrile seizures within 24 h, and 13 with recurrent prolonged febrile seizures (>10-min duration). Twenty-three patients were recruited because of an episode of afebrile status epilepticus, and 12 were recruited because of a single cluster of two or more afebrile seizures within a 24-h period. At the time of recruitment, 129 patients did not have a diagnosis of epilepsy, but by the end of the study period, 49 (38%) of these had acquired an epilepsy diagnosis (Table 1). Seventeen different epilepsy syndrome diagnoses were made. The numbers for epilepsy syndrome diagnosis are shown in Table 2. Of 263 patients, 125 (47.5%) remained without an epilepsy syndrome that could be fully classified. Seventy-six (22.1%) patients in the cohort developed therapy-resistant seizures. Finally, 106 (30.1%) had concerns about development expressed at the time of recruitment, and 115 (33.5%) had developmental concerns raised at most recent follow-up.

## Genetic diagnoses

Three hundred and thirty-three patients completed genetic testing. There was insufficient DNA to complete panel testing for 10 patients. Genetic diagnoses were made for 80 children (24%) (Fig. 1). Supplementary material, part B details all the genetic diagnoses made, along with the phenotypic details for each patient. All but four of the genetic diagnoses were in patients who had a diagnosis of epilepsy by the end of the study period (Table 1). No

patient had more than one diagnostic finding. The incidence of single-gene seizure disorders in this population is at least 1 per 2120 live births (47.2/100 000; 95% CI 36.9–57.5). Twenty-six patients had a diagnostic result from accelerated single-gene testing (Sanger and/or MLPA), therefore, these did not undergo panel testing. Of the remaining 307 patients tested on the panel, 52 patients had a diagnostic result. Finally, two patients, following multidisciplinary team discussion, underwent *KCNQ2* MLPA testing after a negative panel result and were found to have pathogenic copy number variants. The causative variant was *de novo* in 34 patients, inherited from an affected parent in 15 patients, inherited from an unaffected parent in nine patients, undetermined in 21 cases, and compound heterozygous in one patient (with *POLG*-related seizures). Table 2 shows the genetic diagnoses made in relation to phenotype at the end of the study period. High yields from genetic testing were observed in patients classified into the following groups: Dravet syndrome; other developmental and epileptic encephalopathies; self-limited infantile seizures.

The most common single-gene epilepsies were *PRRT2* (17 patients), *SCN1A* (14 patients), *KCNQ2* (10 patients) and *SLC2A1* (seven patients). Eighty-four per cent (67/80) of genetic diagnoses were in the most frequently-implicated 10 genes. Through interrogating clinical data obtained at presentation and follow-up, we were able to characterize the phenotypic spectrum of several single-gene epilepsies (Table 3 and Fig. 2) and define high yield groups for specific single-gene epilepsies (Table 5). In Table 6 we present univariate and multivariate analysis for associations between presenting features and identification of a single-gene cause. Genetic diagnosis was positively associated with presentation before the age of six months, and with presentation with afebrile focal seizures. These associations remained significant in a multivariate model.

## Gene-associated phenotypes

### *PRRT2*: self-limited (familial) infantile seizures

We identified 17 patients and calculate the minimum incidence as 1 per 9970 live births (10.0/100 000; 95% CI

**Table 1** Diagnoses at recruitment and diagnoses at most recent follow-up

Diagnosis at recruitment		Diagnosis at most recent follow-up		% Progression to epilepsy
Group	n	Epilepsy	Not epilepsy	
Recurrent unprovoked seizures (epilepsy)	214	214	0	N/A
Afebrile status	23	13	10	56.5
Afebrile cluster of seizures within 24 h	12	6	6	50.0
Febrile status	45	13	32	28.9
Febrile cluster of seizures within 24 h	36	12	24	33.3
Recurrent prolonged febrile seizures (>10 min)	13	5	8	38.5
<b>Total</b>	<b>343</b>	<b>263</b>	<b>80</b>	
Total (%) with a genetic cause identified	80 (23.3)	76 (28.9)	4 (5.0)	

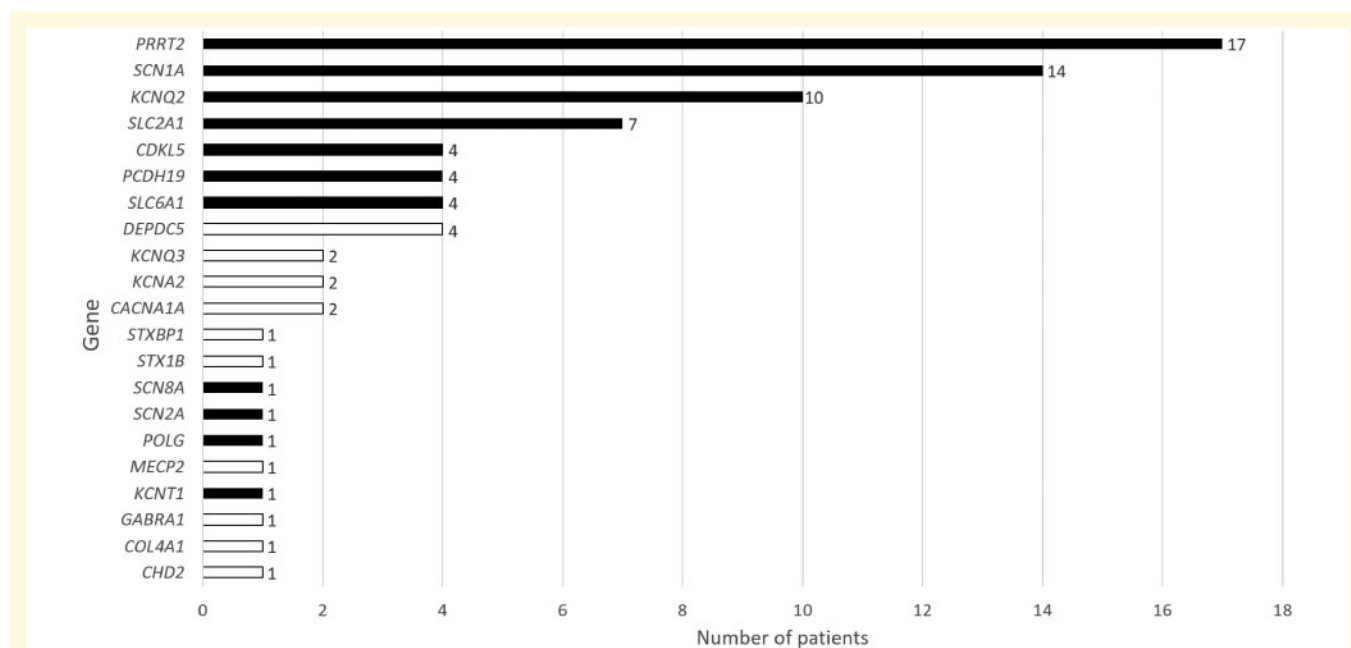
N/A = not applicable.



**Table 2** Final phenotypes and genetic diagnoses made within each group

Phenotype at most recent follow up, <i>n</i>	<i>n</i> (%) with a genetic cause identified	Genes implicated ( <i>n</i> )
<b>Epilepsy, 263</b>		
DEE, 62		
West syndrome, 27	3/27 (11.1)	<i>CDKL5</i> (2), <i>DEPDC5</i>
Dravet syndrome, 11	11/11 (100)	<i>SCN1A</i> (11)
Other DEEs, 24	13/24 (54.1)	<i>PCDH19</i> (3), <i>CDKL5</i> (2), <i>KCNQ2</i> (2), <i>GABRA1</i> , <i>KCNT1</i> , <i>MECP2</i> , <i>SCN2A</i> , <i>SCN8A</i> , <i>STXBP1</i>
Alper-Huttenlocher syndrome, 1	1/1 (100)	<i>POLG</i>
Absences with eyelid myoclonia, 1	1/1 (100)	<i>CHD2</i>
Early onset absence epilepsy, 5	0/5	
Epilepsy with myoclonic-atic seizures, 8	2/8 (25)	<i>STX1B</i> , <i>SLC6A1</i>
Familial focal epilepsy, 1	1/1 (100)	<i>DEPDC5</i>
Febrile seizures plus, 6	0/6	
Genetic epilepsy with febrile seizures plus, 2	1/2	<i>SCN1A</i>
Glut1 deficiency syndrome, 7	7/7 (100)	<i>SLC2A1</i> (7)
Myoclonic epilepsy of infancy, 3	0/3	
Panayiotopoulos syndrome, 1	0/1	
Self-limited familial infantile epilepsy, 5	5/5 (100)	<i>PRRT2</i> (5)
Self-limited infantile epilepsy, 27	11/27 (40.7)	<i>PRRT2</i> (10), <i>KCNQ2</i>
Self-limited familial neonatal seizures, 7	7/7 (100)	<i>KCNQ2</i> (5), <i>KCNQ3</i> (2)
Self-limited neonatal seizures, 1	1/1 (100)	<i>KCNQ2</i>
Unclassified myoclonic epilepsy, 5	0/5	
Unclassified generalized epilepsy, 10	1/10 (10)	<i>CACNA1A</i>
Unclassified focal epilepsy, 59	5/59 (8.5)	<i>DEPDC5</i> (2), <i>KCNA2</i> , <i>KCNQ2</i> , <i>PRRT2</i>
Unclassified focal and generalized epilepsy, 8	0/8	
Unclassified epilepsy, 44	6/44 (13.6)	<i>SLC6A1</i> (3), <i>COL4A1</i> , <i>PCDH19</i> , <i>PRRT2</i>
<b>Not epilepsy, 80</b>		
Febrile seizures only, 64	3/64 (4.7)	<i>SCN1A</i> (2), <i>KCNA2</i> (mosaic, 20)
Single episode of afebrile status, 10	0/10	
Single cluster of afebrile seizures in 24 h, 6	1/6 (16.7)	<i>CACNA1A</i>

DEEs = developmental and epileptic encephalopathies.

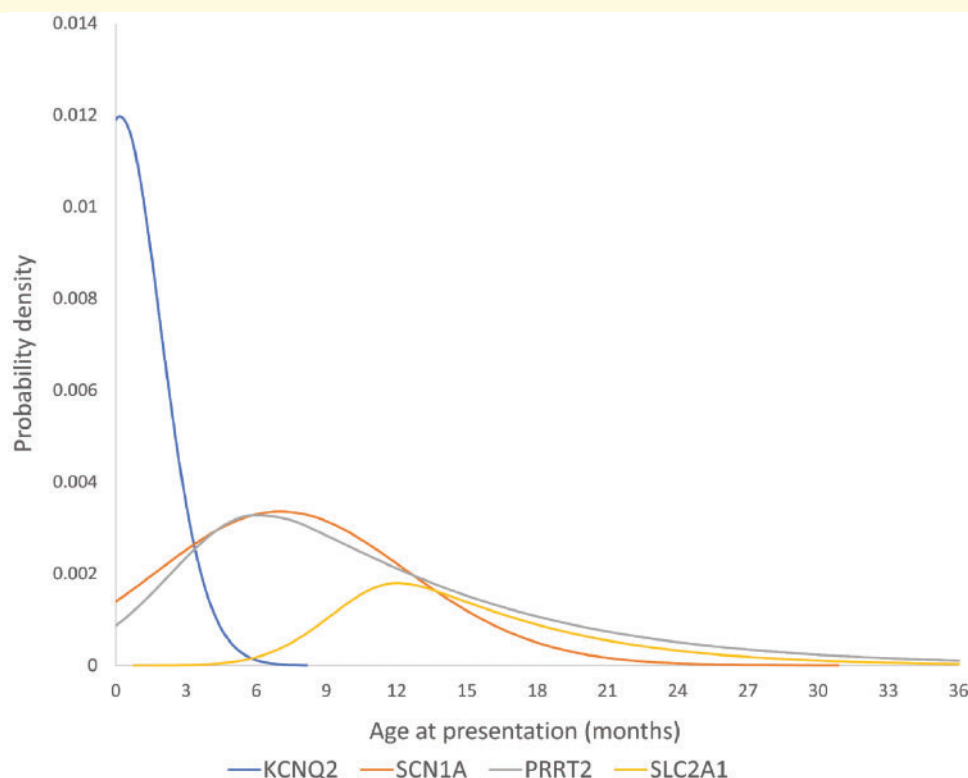
**Figure 1** Genetic results. No case had more than one diagnostic result. Shaded bars represent genes for which there is evidence for precision therapy.

**Table 3** Summary of the clinical findings from the eight most common single-gene epilepsies

	Genetic cause			
	<i>PRRT2</i>	<i>SCN1A</i>	<i>KCNQ2</i>	<i>SLC2A1</i>
Number of patients in this cohort and whether related	17 (8 female) All unrelated	14 (5 female) All unrelated	10 (5 female) All unrelated	7 (3 female) All unrelated
Incidence	1 per 9970 live births (10.0/100 000; 95% CI 5.26–14.8).	1 per 12 200 live births (8.26/100 000; 95% CI 3.93–12.6).	1 per 17 000 live births (5.89/100 000; 95% CI 2.24–9.56).	1 per 24,300 live births (4.13/100 000; 95% CI 1.07–7.19).
Age range at presentation in months (median)	3–19 (7)	1.5–19 (6.5)	0.17–4 (0.24)	11–18 (12)
Most common presentation(s)	Afebrile focal seizures: 71% (12/17)	Febrile seizures: 50% (7/14) Afebrile focal seizures: 36% (5/14) Status epilepticus: 36% (5/14)	Afebrile focal seizures: 70% (7/10)	Afebrile generalized seizures: 86% (6/7)
Diagnosis at latest follow-up	Self-limited infantile epilepsy: 88% (15/17) Unclassified focal epilepsy: 6% (1/17) Unclassified epilepsy: 6% (1/17)	Dravet syndrome: 79% (11/14) Febrile seizures only: 14% (2/14) Genetic epilepsy with febrile seizures plus: 7% (1/14)	Self-limited neonatal epilepsy: 60% (6/10) <i>KCNQ2</i> encephalopathy: 20% (2/10) Self-limited infantile epilepsy: 10% (1/10) Unclassified focal epilepsy: 10% (1/10)	Glut1-deficiency with epilepsy: 100% (7/7)
Developmental concerns at presentation	24% (4/17)	29% (4/14) (29%)	20% (2/10)	57% (4/7)
Developmental concerns at follow-up	12% (2/17)	64% (9/14)	30% (3/10)	43% (3/7)
Therapy-resistant seizures	None (0/17)	86% (12/14)	20% (2/10)	14% (1/7)
Recommended treatment(s) (Table 4)	Carbamazepine	Stiripentol Fenfluramine Cannabidiol Avoidance of sodium channel blocking medications	Carbamazepine Phenytoin	Ketogenic diet
Zygosity	100% heterozygous (17/17)	100% heterozygous (14/14)	100% heterozygous (10/10)	100% heterozygous (7/7)
Inheritance of causative variant	12% <i>de novo</i> (2/17) 71% inherited (12/17) 18% unknown (3/17)	70% <i>de novo</i> (10/14) 30% inherited (3/14) 10% unknown (1/14)	30% <i>de novo</i> (3/10) 50% inherited (5/10) 20% unknown (2/10)	70% <i>de novo</i> (5/7) 15% inherited (1/7) 15% unknown (1/7)

	Genetic cause			
	<i>CDKL5</i>	<i>PCDH19</i>	<i>DEPDC5</i>	<i>SLC6A1</i>
Number of patients in this cohort and whether related	4 (3 female) All unrelated	4 (all female) All unrelated	4 (2 female) All unrelated	4 (all female) 3 were siblings
Incidence	1 per 42 400 live births 2.36/100 000 (95% CI 0.805–5.59)	1 per 20 600 live born females 4.85/100 000 (95% CI 1.97–9.15)	1 per 42 400 live births 2.36/100 000 (95% CI 0.81–5.59)	N/A
Age range at presentation in months (median)	0.5–6 (1.65)	6–18 (11.5)	2.5–26 (20)	12–31 (19)
Most common presentation(s)	Infantile spasms: 50% (2/4) Afebrile focal seizures: 50% (2/4)	Afebrile focal seizures: 75% (3/4)	Afebrile focal seizures: 50% (2/4)	Afebrile focal seizures: 75% (3/4)
Diagnosis at latest follow-up	<i>CDKL5</i> developmental and epileptic encephalopathy: 100% (4/4)	<i>PCDH19</i> related developmental and epileptic encephalopathy: 75% (3/4) Unclassified epilepsy: 25% (1/4)	Unclassified focal epilepsy: 75% (3/4) Infantile spasms (West syndrome): 25% (1/4)	Unclassified epilepsy: 75% (3/4) Epilepsy with myoclonic-atonic seizures: 25% (1/4)
Developmental concerns at presentation	50% (2/4)	None (0/4)	None (0/4)	75% (3/4)
Developmental concerns at follow-up	100% (4/4)	75% (3/4)	25% (1/4)	100% (4/4)
Therapy-resistant seizures	100% (4/4)	100% (4/4)	50% (2/4)	50% (2/4)
Recommended treatment(s) (Table 4)	Ketogenic diet	Clobazam	No specific recommendation	Sodium valproate
Zygosity	75% heterozygous (3/4) 25% hemizygous (1/4)	100% heterozygous (4/4)	100% heterozygous (4/4)	100% heterozygous (4/4)
Inheritance of causative variant	75% <i>de novo</i> (3/4) 25% unknown (1/4)	50% paternally inherited (2/4) 50% <i>de novo</i> (2/4)	50% <i>de novo</i> (2/4) 50% inherited (2/4)	100% inherited (4/4)



**Figure 2 Age of presentation for four genetic epilepsies.** These skewed Gaussian plots are hypothetical distributions based on the mean, median and standard deviations of the age at presentation for these four genetic epilepsies from our data. Each plot has been scaled according to the number of cases identified in this cohort so that the area under each curve represents the total probability of finding a causative variant in each gene in our cohort.

5.26–14.8). Two patients had missense variants and 15 patients had frameshift variants, of whom 13 patients had the recurrent frameshift variant (c.649dup, p.Arg217Profs\*8). This is the most frequently observed disease-associated *PRRT2* variant in the literature (Ebrahimi-Fakhari *et al.*, 2015). The observation that this variant appears over 400 times in the exome aggregation consortium (ExAC) database (Lek *et al.*, 2016) would initially suggest that it is a relatively common population variant. However, the same variant appears just eight times in the genome aggregation consortium (gnomAD) database, which includes the same participants as ExAC but applies a different sequencing methodology (The Broad Institute, 2018). We therefore suspect that the variants seen in ExAC are artefacts that have appeared during the DNA amplification process. In our study we validated all variants through Sanger sequencing and are confident that we have not reported sequencing artefacts. Among 29 718 participants in gnomAD for whom there is *PRRT2* data, eight were heterozygous for the c.649dup variant. In our study there were 10 patients among 333 tested, making this variant >100 times more common in our study population than in the healthy population.

A clear age pattern of *PRRT2*-related epilepsy presentation was observed, with peak onset of seizures at 7 months.

The phenotype we observed was in keeping with previously published literature on *PRRT2*-related epilepsy, which describes a self-limited infantile epilepsy which has median age of onset at 6.0 months (Ebrahimi-Fakhari *et al.*, 2015). Outcomes were generally good in our cohort. At most recent follow-up no patients had developed therapy-resistant seizures, and all had been seizure-free for >6 months. Interestingly four patients had developmental concerns highlighted at presentation but only two continued to present developmental concerns at most recent follow-up. This provides a suggestion that with this genetic epilepsy, developmental trajectory may improve along with seizure control as the child gets older. This hypothesis would need to be confirmed by studying more prospective developmental assessments. Effective therapies reported were carbamazepine ( $n = 7$ ), levetiracetam ( $n = 5$ ), and sodium valproate ( $n = 3$ ).

### **SCN1A: Dravet syndrome, febrile seizures plus**

We identified 14 patients and calculate the minimum incidence of *SCN1A*-related seizures as 1 per 12 200 live births (8.26/100 000; 95% CI 3.93–12.6). A spectrum of disease severity was seen. Of 14 patients with *SCN1A* variants, 11 (79%) were ultimately diagnosed with Dravet syndrome, making the incidence of *SCN1A*-related Dravet syndrome

**Table 4** Evidence from the literature to support gene-specific treatment approaches

Gene	Recommendation(s)	Evidence base					Reference
		n	Study details	P-value	Evidence level	Recommendation grade	
PRRT2	Consider carbamazepine	64	Retrospective uncontrolled clinician-reported subjective treatment response analysis.	NC	III	C	Huang et al. (2015)
		24	Retrospective uncontrolled clinician-reported subjective treatment response analysis.	NC	III		Ebrahimi-Fakhari et al. (2015)
SCN1A	Consider stiripentol	36	Placebo-controlled RCT of add-on therapy in Dravet syndrome (number with SCN1A variant not reported).	<0.0001	IB	A	Chiron et al. (2000)
		41	Prospective observational study of long-term efficacy of add-on stiripentol in patients with Dravet syndrome (39 with SCN1A variant).	NC	III		Myers et al. (2018)
	Consider cannabidiol	120	Multicentre double-blinded placebo-controlled RCT of add-on therapy in Dravet syndrome (114 with SCN1A variant).	0.08	IB	A	Devinsky et al. (2017)
	Consider fenfluramine	11	Retrospective uncontrolled clinician-reported seizure-freedom.	NC	III	C	Ceulemans et al. (2012)
	Consider ketogenic diet	20	Retrospective uncontrolled clinician-reported seizure reduction in Dravet syndrome (number with SCN1A variant not reported).	NC	III	C	Caraballo et al. (2005)
	Consider levetiracetam	28	Open label uncontrolled trial of add-on lamotrigine in Dravet syndrome (16 with SCN1A variant).	0.0001	III	C	Striano et al. (2007)
	Consider topiramate	18	Open label uncontrolled trial of add-on topiramate in Dravet syndrome (number with SCN1A variant not reported).	NC	III	C	Coppola et al. (2002)
	Consider sodium valproate	160	Retrospective uncontrolled clinician-reported subjective treatment response analysis.	NC	III	C	Brunklaus et al. (2012)
	Avoid carbamazepine	60	Retrospective uncontrolled clinician-reported subjective treatment response analysis.	NC	III	C	Brunklaus et al. (2012)
	Avoid lamotrigine	60	Retrospective uncontrolled clinician-reported subjective treatment response analysis.	NC	III	C	Brunklaus et al. (2012)
		21	Uncontrolled unblinded trial of add-on lamotrigine in Dravet syndrome (number with SCN1A variant not reported).	NC	III		Guerrini et al. (1998)
KCNQ2	Consider carbamazepine	15	Retrospective uncontrolled clinician report of seizure-freedom.	NC	III	C	Pisano et al. (2015)
	Consider phenytoin	15	Retrospective uncontrolled clinician report of seizure-freedom.	NC	III	C	Pisano et al. (2015)
SLC2A1	Use ketogenic diet	104	Retrospective uncontrolled family-reported subjective treatment response analysis.	NC	III	C	Kass et al. (2016)
CDKL5	Consider ketogenic diet	82	Retrospective uncontrolled family-reported subjective treatment response analysis.	NC	III	C	Lim et al. (2017)
PCDH19	Consider clobazam	58	Retrospective uncontrolled clinician-reported treatment response analysis, 3 months after commencing therapy.	NC	III	C	Lotte et al. (2016)
SLC6A1	Consider sodium valproate	15	Retrospective uncontrolled clinician-reported treatment response analysis.	NC	III	C	Johannessen et al. (2018)
SCN8A	Consider phenytoin	0	Functional study. Single cell patch clamp testing in ND/7 cells transfected with the epilepsy-associated variant (I1327V).	NC	III	C	Barker et al. (2016)
		4	Retrospective uncontrolled clinician-reported subjective treatment response analysis.	NC	III		Boerma et al. (2016)
SCN2A (with seizure onset <3 mo of age)	Consider sodium channel blocking (SCB) drugs	158	Retrospective clinician-reported seizure-freedom.	$1 \times 10^{-6}$	III	C	Wolff et al. (2017)
POLG	Avoid sodium valproate	43	Retrospective clinician-reported hepatotoxicity.	NC	III	C	Anagnostou et al. (2016)
KCNIT1 (in patients aged <4 y)	Consider trial of quinidine	0	Functional study. Single cell patch clamp testing in <i>Xenopus laevis</i> oocyte cells.	NC	III	None (conflicting evidence)	Milligan et al. (2014)
		6	Single centre, inpatient, order randomized, blinded, placebo-controlled trial.	0.15	NA		Mullen et al. (2018)

Papers were included if they were either randomized-controlled trials, provided supportive evidence from *in vitro* functional studies, or analysed a cohort of > 10 patients specifically in relation to treatment response. Where multiple treatments were evaluated in the same cohort, evidence is presented in favour of the most efficacious therapy identified. See Supplementary material for a more detailed version of this table.

mo = months; NC = not calculated; y = years.

1 per 15 500 live births. One patient, who had a *de novo* variant (Patient 90), received a diagnosis of genetic epilepsy with febrile seizures plus (GEFS+). *De novo* SCN1A variants do not necessarily imply a severe prognosis and have previously been associated with GEFS+ phenotypes (Myers et al. 2017). Two patients had febrile seizures only, one of whom (Patient 5) had an inherited variant from a father

with a history of recurrent febrile seizures. For the other (Patient 317), inheritance status could not be determined. Six patients had variants predicted to result in truncation and eight had missense variants, but no genotype-phenotype association was observed in this cohort. One of the patients with a truncating variant (Patient 5) had febrile seizures only. Age at presentation had a similar distribution to that



**Table 5** Presentation types that had high yield for specific genetic diagnoses

Presentation <i>n</i>	Afebrile seizures < 6 months 63	Afebrile focal seizures < 12 months 68	Febrile or afebrile status epilepticus <24 months 59	Afebrile generalized seizures ≥ 6 months and <24 months 74
Genetic diagnosis ( <i>n</i> )	<b>KCNQ2 (10)</b> PRRT2 (5) SCN1A (4) CDKL5 (2) KCNQ3 (2) COL4A1 (1) GABRA1 (1) KCNT1 (1) STCBP1 (1) SCN2A (1) SCN8A (1)	<b>PRRT2 (11)</b> KCNQ2 (7) SCN1A (5) CDKL5 (2) KCNQ3 (2) COL4A1 (1) DEPDC5 (1) GABRA1 (1) PCDH19 (1) SCN2A (1) SLC2A1 (1)	<b>SCN1A (5)</b> KCNA2 (1) POLG (1) PRRT2 (1) SLC2A1 (1)	<b>SLC2A1 (6)</b> PRRT2 (2) CHD2 (1) KCNA2 (1) PCDH19 (1) POLG (1) SCN1A (1)
Total with genetic diagnosis (%)	<b>29 (46.0)</b>	<b>33 (48.5)</b>	<b>9 (15.2)</b>	<b>13 (17.6)</b>

**Table 6** Associations between features at presentation and genetic diagnosis

	<i>n</i> (%) with genetic cause identified	Two-tailed Fisher's exact probability <sup>ψ</sup>	OR (95% CI)	Multivariate model probability (Hosmer- Lemeshow)	Multivariate OR (95% CI)
<b>Total cohort</b>	80/333 (24.0)				
<b>Age at presenting seizure</b>					
< 6 months	34/74 (45.9)	< 0.005**	3.9 (2.3–6.9)	0.004	4.9 (1.9–12.8)
6–12 months	22/89 (24.7)	n.s.		n.s.	
12–24 months	17/117 (14.5)	< 0.005*	0.4 (0.2–0.7)	n.s.	
24–36 months	7/53 (13.2)	n.s.		Reference category	
<b>Presenting seizure type</b>					
Febrile generalized, not including status	2/36 (5.6)	< 0.005*	0.2 (0.0–0.7)	Reference category	
Febrile focal, not including status	3/10 (33.3)	n.s.		n.s.	
Febrile status, generalized or focal	7/45 (15.6)	n.s.		n.s.	
Afebrile focal, not including status	40/100 (40.0)	< 0.005**	3.2 (1.9–5.3)	0.012	6.9 (1.5–31.7)
Afebrile generalized, not including status <sup>‡</sup>	21/96 (21.9)	n.s.		n.s.	
Afebrile status, generalized or focal	3/21 (14.3)	n.s.		n.s.	
Afebrile unclassified	1/4 (25.0)	n.s.		n.s.	
Infantile spasms	3/21 (14.3)	n.s.		n.s.	
Afebrile generalized tonic-clonic	9/50 (18.0)	n.s.		Subtypes of afebrile generalized seizures were not included in the multivariate model	
Afebrile generalized myoclonic	4/23 (17.4)	n.s.			
Afebrile generalized tonic	4/9 (44.4)	n.s.			
Afebrile generalized atonic	1/3 (33.3)	n.s.			
Afebrile generalized absence	3/11 (27.3)	n.s.			

<sup>ψ</sup>Fisher's exact statistic calculated on a contingency table where the null hypothesis was that there would be equal proportions of patients with and without a genetic diagnosis in each subgroup as there were in the entire tested cohort who completed genetic testing (*n* = 333).

\*Negative association.

\*\*Positive association.

<sup>‡</sup>Composite group of all presentations with afebrile generalized seizures.

n.s. = not significant; OR = odds ratio.

of the *PRRT2*-related seizures, with median onset at 6.5 months. In contrast to patients with *PRRT2* variants, those with *SCN1A* variants were likely to present with febrile seizures and with status epilepticus. Half of the *SCN1A* patients presented with a febrile seizure and half with an afebrile seizure. The *SCN1A* phenotype observed in our

cohort was in line with the previous literature on this genetic seizure disorder, which reports a tendency for early seizures to be associated with fever, a median initial seizure presentation at 6.0 months and a spectrum of disease severity. While the majority go on to develop a drug-resistant epilepsy and cognitive stagnation or decline (Harkin *et al.*, 2007;

Brunklaus *et al.*, 2012) some, even if their variant has arisen *de novo*, may have mostly febrile seizures and a good cognitive outcome (Myers *et al.*, 2017). While it is well established that there is a spectrum of *SCN1A*-related phenotypes, until now the relative proportions of mild versus severe cases has not been established because no study has used a population-based approach to *SCN1A* testing. Here we show that the majority of *SCN1A* phenotypes are in fact at the severe end of the spectrum. In our cohort, 11/14 patients were reported to have normal development at the time of presentation. At most recent follow-up five continued to have normal cognitive development, three had mild cognitive concerns and six had moderate cognitive concerns. Eighty-six per cent (12/14) of patients developed therapy-resistant seizures, making *SCN1A* the most common genetic cause of therapy resistant seizures in this cohort. The most frequently reported effective therapy was stiripentol ( $n = 4$ ).

### **KCNQ2: self-limited (familial) neonatal seizures, early infantile developmental and epileptic encephalopathy**

We identified 10 patients and calculate the minimum incidence as 1 per 17 000 live births (5.89/10 000; 95% CI 2.24–9.56). Seizures associated with *KCNQ2* variants presented significantly earlier than the other single-gene epilepsies in this cohort (median 7 days). Five patients had a frameshift variant, two had a duplication of exons 2–12, two had whole gene deletions, and one had a *de novo* missense variant. The most severe epilepsy and developmental impairment was observed in the patient with the missense variant. The literature describes two distinct phenotypes associated with *KCNQ2* variants: a self-limited familial neonatal seizure phenotype (Biervert *et al.*, 1998; Singh *et al.*, 2003) and a severe neonatal-onset developmental and epileptic encephalopathy (Weckhuysen *et al.*, 2012; Kato *et al.*, 2013; Olson *et al.*, 2017). We also observed these two phenotypes but found that the majority of patients had a mild phenotype. Seven of 10 patients (70%) had self-limited neonatal or infantile-onset seizures, all of whom had normal cognitive development at most recent follow-up. Two patients had *KCNQ2* encephalopathy, and one patient had an unclassified drug-resistant focal epilepsy of onset at 3 months and developed mild cognitive impairment.

Previous studies have identified that more severe phenotypes are observed in patients who carry missense variants in *KCNQ2* (Weckhuysen *et al.*, 2012; Kato *et al.*, 2013; Olson *et al.*, 2017). In contrast, the majority of familial self-limited cases are associated with truncating variants (Singh *et al.*, 2003). We did not entirely observe this pattern. In our cohort only one patient had a missense variant (Patient 177). This patient had a severe developmental and epileptic encephalopathy, presenting with seizures at 30 days of age. The other patient with a severe developmental and epileptic encephalopathy had a whole gene deletion. We observed a number of patients presenting with focal seizures beyond the neonatal period (one at 3 months

and two at 4 months). Post-neonatal presentation with *KCNQ2*-related seizures has been reported before (Millichap *et al.*, 2017; Zeng *et al.*, 2018). In our cohort early onset of seizures appeared to be associated with better outcomes. All six of those who presented at under 1 month of age had self-limited seizures and normal cognitive development. Inheritance status did not fully correlate with severity of phenotype. One patient with an inherited variant and an extensive family history of self-limited familial neonatal seizures had profound cognitive impairment (Patient 177), suggesting modifying factors were at play. Another patient (Patient 336) with a *de novo* variant, had self-limited neonatal seizures and a good cognitive outcome.

The most frequently-reported effective therapy was carbamazepine ( $n = 4$ ), followed by phenobarbitone ( $n = 3$ ).

### **SLC2A1: generalized seizures with gait ataxia (Glut1 deficiency)**

We identified seven patients and calculate the minimum incidence as 1 per 24 300 live births (4.13/100 000; 95% CI 1.07–7.19). Four patients had truncating variants and three had missense variants. In contrast to all the other genetic causes, these patients were more likely to present with generalized seizures than with focal seizures. Six of seven patients presented with generalized seizures (three tonic-clonic, two myoclonic, one absence). Age at presentation was later than that observed with the other genetic causes, with a median seizure-onset of 12 months. This is slightly later than the median age of 8 months reported by Pong *et al.* (2012) in their study of 78 patients with *SLC2A1*-related seizures. The literature on *SLC2A1*-related phenotypes tends to emphasize the coexistence of early childhood seizures with other clinical features, most notably developmental delay, chorea, dystonia and microcephaly (Di Georgis and Veggiotti, 2013). Nonetheless, the observation that initial seizure presentation in these children is often with absence seizures or myoclonic seizures (Hully *et al.*, 2015) prompted some authors to screen children with early onset absence epilepsy (EOAE) and epilepsy with myoclonic atonic seizures (EMA), for pathogenic variants in the *SLC2A1* gene. The conclusions from these studies was that glucose 1 transporter (Glut1) deficiency was associated with a significant minority of these presentations, 12% (Arsov *et al.*, 2012) and 5% (Mullen *et al.*, 2011), respectively. In the seven patients with causative *SLC2A1* variants in our cohort only two had myoclonic seizures at any time in their history, and only one has absence seizures. The most frequent presenting seizure type was generalized tonic clonic seizures, an observation supported by the study by Pong *et al.* (2012), which reported that a generalized tonic clonic seizure was the presenting seizure in 53% of patients with *SLC2A1*-related seizures.

Beyond the seizures, additional phenotypic features at initial presentation were uncommon. In only two patients was Glut1 deficiency suspected prior to genetic result and only one patient underwent diagnostic lumbar puncture. One patient had a marked four limb dystonia at presentation. All the others were thought to have normal motor

function at the time of their seizure presentation. By the time their genetic result was known, and they were clinically re-reviewed, all six of these had developed a subtle gait ataxia. All seven patients are currently on the ketogenic diet, the established treatment of choice for Glut1 deficiency. Ketogenic diet was perceived to be effective in all cases. At most recent follow-up all seven patients were seizure-free. Four had normal cognition and three had mild cognitive concerns. Long-term follow-up of this cohort and comparison with historical cohorts may help determine whether early instigation of the ketogenic diet is associated with improved outcomes.

## Therapy-response and precision therapy:

Seventy-six patients developed therapy resistant seizures, 36 of whom (47%) had a single-gene cause identified. Therapy-resistance was not observed in any patients with *PRRT2* variants, and was seen in only 2/10 patients with *KCNQ2* variants. Just one patient with *SCL2A1*-related seizures developed therapy-resistance. The good seizure outcome observed in Glut1 deficiency may be related to early establishment of the ketogenic diet. The only single-gene epilepsies for which more than two patients developed therapy-resistance were *SCN1A* (12 patients), *CDKL5* (four patients), and *PCDH19* (four patients). Between them, these three genes accounted for 20/76 (26%) of all therapy-resistant cases in this cohort. Literature review identified evidence to support specific treatment approaches for 64 (80%) of our 80 children with genetic diagnoses (Table 4).

## Discussion

In this study we have used a whole population prospective cohort design to determine the incidence of the more common single-gene epilepsies of early childhood. Recruitment to our cohort was consistent across all 24 centres, which represent all health facilities where young children are expected to present with seizures. Patient ascertainment and exclusion of duplicate reporting were well managed in the recruitment process by cross-referencing within clinical departments, attending physicians, specialist epilepsy nurses, EEG departments and the central genetic laboratory.

The panel of 104 established epilepsy-associated genes was designed to capture all the more commonly-implicated genes, with a specific focus on those for which precision treatment approaches exist. As some genetic epilepsies initially present with prolonged febrile seizures (Brunklaus *et al.*, 2012; Higurashi *et al.*, 2013; Ebrahimi-Fakhari *et al.*, 2015) children with status, clusters of febrile seizures, and recurrent prolonged febrile seizures were included.

The approach to determination of diagnostic genetic results involved comprehensive review of genetic and

phenotype data within a multidisciplinary environment. Genetic results were reported in accordance with UK best practice guidelines (Association for Clinical Genetic Science, 2017). Where DNA samples and/or phenotype details from other family members were considered relevant to variant interpretation these were requested. We agree with Anderson and Lassmann (2018) that variants cannot be considered in isolation from phenotypes and relevant variant interpretation requires a multidisciplinary approach.

The incidence of epilepsy in children under 36 months of age has previously been estimated in a US population-based cohort as 1 per 613 live births (Wirrell *et al.*, 2012). Our cohort represents 1 per 495 live births in Scotland. Direct comparison between these cohorts is not appropriate as our study included children presenting with certain febrile seizure presentations and excluded those with established non-genetic causes. In our cohort, 163 patients presented with recurrent afebrile seizures before the age of 12 months, giving an estimated incidence of infantile-onset epilepsy of 1 per 1041 live births (96.0/100 000; 95% CI 81.8–112.0). This is comparable to the figure of 1 per 1240 live births derived from a 20-year population-based cohort in Helsinki (patients with structural-metabolic causes removed) (Gaily *et al.*, 2016) and the figure of 1 per 1220 from a North London cohort (Eltze *et al.*, 2013).

It is established that single-gene aetiology can be identified in a substantial proportion of patients presenting with early-childhood onset epilepsy. Berg *et al.* (2017) carried out a prospective study in which they aimed to determine aetiology in all patients with epilepsy presenting before the age of 3 years, regardless of severity. Participants were recruited from 17 epilepsy centres in the USA (Berg *et al.*, 2017). Their study was not population-based and used a variety of testing methods that were not consistent between centres. They reported a diagnostic yield of 29.2% for those tested on epilepsy gene panels, and 27.8% for those tested by whole-exome sequencing. These yields are slightly higher than in our study. In Berg *et al.*'s study the majority of patients (266/446) without determined aetiology did not undergo any form of genetic testing so those results are likely to have been affected by a degree of ascertainment bias.

In comparison with some recently published studies of genetic testing in epilepsy (Heyne *et al.*, 2018; Lindy *et al.*, 2018) ours includes a smaller cohort of patients. The strength of the present study relates to case ascertainment. The broad inclusion criteria and proactive recruitment strategy applied allowed a better understanding of both the full phenotypic spectrum and the incidence of the single-gene epilepsies in childhood. For the most frequently encountered single-gene epilepsies in this cohort—namely, *PRRT2*, *SCN1A*, *KCNQ2* and *SLC2A1*—we observed phenotypic spectra that were largely in keeping with the literature published previously. However, we demonstrated that these are more common than has been previously described. Previous reports have estimated the incidence of *SCN1A*-related Dravet syndrome in

California (1 per 20 900 live births) (Wu *et al.*, 2015) and in Denmark (1 per 22 000 live births) (Bayat *et al.*, 2015) but neither study used prospective case ascertainment strategies. Our study estimates the incidence of *SCN1A*-related Dravet syndrome to be 1 per 15 500 live births (11 patients with *SCN1A*-related Dravet syndrome). The incidence of Glut1 deficiency has previously been estimated as 1 per 90 000 live births in Queensland (Coman *et al.*, 2006) and 1 per 83 000 live births in Denmark (Larsen *et al.*, 2015) compared with 1 per 24 300 live births in this study. These figures are not likely to represent a Scottish population-specific phenomenon since the majority of cases of Dravet syndrome and Glut1 deficiency are caused by *de novo* variants. Estimated incidences for *PRRT2*, *CDKL5*, *DEPDC5*, and *PCDH19*-related epilepsies are provided here for the first time.

Previous studies investigating the yield of NGS in epilepsy have found that the majority of diagnoses are concentrated in a small number of recurrently-implicated genes. Lindy *et al.* reported the results of testing >8500 patients using a 70-gene epilepsy panel. They quoted a yield of 15.4% (Lindy *et al.*, 2018). As with our study >80% of their diagnoses were in the most frequently-implicated seven genes. Six of our seven most frequently-implicated genes were among their seven most frequently-implicated (*DEPDC5* was not included in their panel). We identified a substantially higher rate of *PRRT2* variants in this cohort than have been reported in previous studies (Helbig *et al.*, 2016; Trump *et al.*, 2016; Lindy *et al.*, 2018). This is likely to reflect our inclusion of self-limited and pharmaco-responsive epilepsies that would not have previously been considered candidates for high throughput genetic testing.

For a number of reasons this study is likely to have underestimated the incidence of single-gene epilepsies in this group, so our incidence figures are best considered as minimum incidences. Some genes associated with epilepsy are not on our panel, and for some genes (e.g. *SCN1A* and *KCNQ2*) we were able to offer more comprehensive testing than for others through MLPA. Chromosomal microarray for deletions and duplications was not part of our routine testing strategy due to the low reported yield in this group, low penetrance of many epilepsy-associated variants, and absence of evidence that identification of chromosomal lesions supports a precision medicine approach. Though the inclusion criteria for our study were broad, we are likely to have missed some patients with very mild phenotypes—e.g. recurrent simple febrile seizures—who did not meet eligibility criteria. The identical twin of Patient 334 had the same *PRRT2* variant as her sister but was not eligible for inclusion since all her seizures were febrile and <10 min duration. Similarly, *SCN1A*-related disease can present with simple febrile seizures only (Escayg *et al.*, 2000). A complex febrile seizure has been defined by some authors as a febrile seizure that has any one of the following elements: focal features, duration >15 min, recurring more than once in

24 h, or associated with postictal palsy or previous neurological deficits (Capovilla *et al.*, 2009). Our criteria did not include children with febrile seizures with focal features lasting <10 min or those with a single febrile seizure lasting between 10 and 30 min as duration and frequency of febrile seizure were considered more reliable clinical predictors of *SCN1A*, *PCDH19* and *PRRT2* variants.

We are likely to have underestimated the incidence of *SLC2A1*-related disease as not all patients with Glut1 deficiency will present with seizures in the first 3 years of life. The same is true for several other genetic epilepsies and neurodevelopmental disorders, including *CDKL5*, *DEPDC5*, *POLG*, *SCN2A*, *MECP2*, *KCNT1* and *GABRA1*. According to our protocol patients were not recruited to the study if they had an aetiology identified either prior to or at initial presentation with seizures. As a result, patients are likely to have not been recruited if they had acute neuroimaging findings that were deemed to explain their epilepsy, even if such findings may indeed have an underlying genetic basis. The most notable example of this would be tuberous sclerosis, caused by *TSC1/TSC2* variants, where neuroimaging findings are often highly typical for this genetic disorder and the diagnosis may be known prior to onset of seizures. Patients with other genetically determined developmental brain malformations may also not have been recruited. Neonates with symptomatic seizures secondary to hypoxic ischaemic encephalopathy (HIE) at birth were not recruited. Rarely, genetic metabolic disorders such as pyridoxine dependency, sulphite oxidase deficiency, or molybdenum co-factor deficiency may mimic HIE (Baxter, 1999). We would expect these children to continue to have seizures beyond the neonatal period and to be seen within one of Scotland's tertiary child neurology centres. Finally, we only tested DNA samples derived from blood and it has been shown that some genetic epilepsies are due to somatic variants (Nellist *et al.*, 2015).

Evidence from randomized controlled trials (RCTs), open label trials, retrospective case series, and from *in vitro* functional studies, informs clinicians' treatment choice when they make a diagnosis of a single-gene epilepsy. Such evidence exists for *SCN1A*, *PRRT2*, *KCNQ2*, *SLC2A1*, *PCDH19*, *POLG*, *SCN2A*, and *SCN8A* (Table 4). On this basis we estimate that 64/80 (80%) of the single-gene diagnoses made in this study had potential treatment implications. In the case of Glut1 deficiency, early diagnosis and implementation of the ketogenic diet may have an impact on developmental and motor comorbidity as well as seizure control (Kass *et al.*, 2016). In *SCN1A*-related epilepsy, duration of use of contraindicated sodium-channel blocking medication is associated with adverse developmental outcome (de Lange *et al.*, 2018). Additional benefits of early genetic diagnosis in epilepsy include providing information for genetic counselling (Krabbenborg *et al.*, 2016), giving answers for affected families (Brunklaus *et al.*, 2013; Sawyer *et al.*, 2016; Wynn *et al.*, 2018), and avoidance of



additional costly and invasive investigations. Recent economic analyses have demonstrated that the application of early high throughput genetic testing could save \$5236 Australian dollars (Palmer *et al.*, 2018), or \$7047 US dollars (Howell *et al.*, 2018) per diagnosis when compared with investigation programs that involved extensive imaging and metabolic testing prior to genetic testing.

Evidence in support of precision therapy in epilepsy varies in level and nature. In 2019, the majority of truly medically ‘actionable’ genetic diagnoses in epilepsy relate to inherited disorders of metabolism such as Glut1 deficiency, and pyridoxine dependency (Peng *et al.*, 2019). Questions remain unanswered in relation to targeted treatment of other genetic causes of epilepsy. Much of the evidence that we have presented to support gene-specific therapy approaches in Table 4 is at level III. Non-RCT evidence is compromised by the absence of control groups, variability in timing and objectivity of response analysis, and inconsistent reporting of concomitant drug use. As exemplified by *KCNT1*-related seizures, evidence can be conflicting. Here, despite positive anecdotal reports of benefit from quinidine (Bearden *et al.*, 2014; Fukuoka *et al.*, 2017; Abdelnour *et al.*, 2018) and supportive *in vitro* functional studies (Milligan *et al.*, 2014) a small randomized-controlled crossover trial demonstrated no significant benefit in adult patients with *KCNT1*-related frontal lobe epilepsy (Mullen *et al.*, 2018). Nevertheless, in reality, many clinicians if faced with a child with unremitting seizures associated with a *KCNT1* variant may be inclined to at least give a trial of quinidine—a drug that they would be unlikely to use for seizures in any other scenario. The recommendation grade for specific therapy in most of the genetic epilepsies is grade C. Nonetheless it is important to note that such evidence is the basis of therapy choice in almost all epilepsy syndromes and may provide a key lead in to definitive trials, as has been the case with fenfluramine in Dravet syndrome (NIH US National Library of Medicine, 2019). Findings from RCTs must also be interpreted in context. In Dravet syndrome cannabidiol demonstrates efficacy (Devinsky *et al.*, 2017); however, there is no biological reason why cannabidiol should be specifically effective in this condition because it does not appear to act on sodium channels or GABA receptors (Devinsky *et al.*, 2014). Cannabidiol also has efficacy in Lennox Gastaut syndrome (French *et al.*, 2017; Devinsky *et al.*, 2018), an epilepsy with varied aetiologies. Several other broadly-acting anti-epileptic therapies including levetiracetam, topiramate, and the ketogenic diet have performed just as well as cannabidiol in open label uncontrolled studies of Dravet syndrome, but as they have not been tested in RCT format they are considered less evidence-based (Coppola *et al.*, 2002; Caraballo *et al.*, 2005; Striano *et al.*, 2007; Devinsky *et al.*, 2018). In contrast the ketogenic diet is regarded as the gold standard therapy in Glut1 deficiency in the absence of any RCT data. Obtaining good

quality evidence for gene-specific treatment approaches in epilepsy is perceived as a challenge, since many of these disorders are exceedingly rare. To this end, defining the incidence of the more common difficult to treat genetic epilepsies of childhood is an important step. Orphan medicinal products have been developed and licensed for many rarer conditions than the genetic epilepsies (European Joint Programme Rare Diseases, 2019). Gene therapy approaches, which may provide definitive precision therapy, are being trialled in rodent and non-human primate models of human genetic epilepsies (Berkovic *et al.*, 2015). In this study, 36/80 patients with single-gene epilepsy had therapy-resistant seizures. Of these 20 (56%) were associated with just three genes, *SCN1A*, *CDKL5*, and *PCDH19*. For maximum benefit, these are the genetic epilepsies that should be prioritized in the development of precision therapy.

## Study limitations

Although we aimed to include all children with epilepsy presenting in Scotland under the age of 3 years, it is possible that some patients were unreported and not included. Therefore, all incidences reported in this study should be viewed as minimum estimates.

Children with epilepsy due to other identifiable causes such as hypoxic ischaemic encephalopathy, meningitis, metabolic disorders, etc. were excluded. However, some of these patients may have had genetic ‘mimics’ of acquired causes, genetic causes of structural brain abnormalities, or compound genetic-acquired aetiologies. Their exclusion may have reduced the yield of genetic diagnoses in this study.

The health economics of genetic testing in this group of children were not examined, but it is possible that early identification of a genetic diagnosis would save other costly investigations.

This cohort is being followed up to determine whether there is a positive impact of early genetic diagnosis and treatment on a child’s neurodevelopment and comorbidities. However, this study will take several years to report its conclusions.

## Conclusions

Single-gene epilepsies are more common than previously reported, with a collective minimum incidence of about 1 per 2000 live births. Many of the cases identified in this study are dominant genetic epilepsies due to *de novo* mutations. Therefore these minimum incidence figures are applicable to other populations and are not specific for Scotland.

Our data suggest that genetic testing should be a primary investigation for epilepsies presenting in early childhood. The nature of genetic testing will depend upon available



resources. Eighty per cent of genetic diagnoses in this group relate to eight genes, with other genetic aetiologies likely to be individually extremely rare. A clinically relevant and economically efficient testing paradigm would be to analyse a small panel of genes and if this is unrevealing move to a larger platform such as clinical exome, whole exome or whole genome.

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## Competing interests

The authors report no competing interests.

## Supplementary material

Supplementary material is available at *Brain* online.

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